Lumiprobe
Life science solutions

Protein Labeling with 3Dye Differential Proteomic Reagents

Differential proteomics allows comparison of big sets of proteins originating from different biological sources. The usual examples are treated and untreated cells, different bacterial strains. Even small differences in protein profiles can be discovered by differential proteomics.

Lumiprobe 3Dye Kit contains Cyanine2, Cyanine3, and Cyanine5 dyes, which are spectrally distinct but mobility-matched. Thus, proteins labeled with these dyes co-migrate in gel-electrophoresis. Because protein labeling with the dyes relies on the NHS ester chemistry, lysine residues are predominant sites of the labeling.

The best practice of 2D proteomics implies the use of a pooled internal control sample which is essentially a mixture of two samples that will be analyzed in one experiment. The internal control sample is labeled with Cyanine2, and the samples for analysis are labeled with Cyanine3 or Cyanine5 (these two dyes are interchangeable).

3Dye reagents come pre-measured in 5 nmol, 10 nmol, and 25 nmol packages. Dyes are lyophilized to prolong their shelf life. Each package should be re-constituted with DMF prior to use. The DMF quality is essential for the experiments and the product shelf life. DMF comes with the dyes in the kit.

Protein mixture preparation is the most variable and demanding part of the assay. It is outside the scope of the protocol because different samples require very different lysis conditions to obtain a mixture of proteins suitable for the assay. Essentially, both samples being compared should be lysed in identical conditions to achieve meaningful results. Generally, if experiment requires lysis, CHAPS-based lysis solutions are recommended.

After the preparation of both protein mixtures being compared, the following protocol can be used to achieve labeling:

1. Prepare 1 mM stock solution of each dye by adding 1 µL of DMF (from the kit) per 1 nmol of each dye. These solutions are stable for three months at -20°C. To ensure shelf life, desiccate, completely unfreeze the tubes before opening, purge with inert gas when possible before closing.
2. Adjust protein mixtures pH to 8.5 by using either amine-free buffer (NaHCO3, acetate) or Tris buffer. In separate vials, prepare three protein samples for labeling: (1) the first protein sample, (2) the second protein sample, and (3) the mixture of both (pooled internal control). Protein concentration in each sample should ideally be 5–10 mg/mL, but as low as 1 mg/mL can be used. Take 50 µg of protein per reaction.
3. Prepare working solutions of dyes by taking aliquots of 1 mM stock solution and diluting with DMF to 0.4 mM.
4. Add 1 µL of working dye solution to reaction mixture: Cyanine3 for untreated, Cyanine5 for treated (or vice versa), and Cyanine2 for pooled internal control. Mix each reaction by pipetting it in and out and leave for 30 min in the dark.
5. Stop the reactions by adding 1 µL of 10 mM lysine to each solution (it is not included in the kit, but this reagent is readily available).
6. Pool the three samples and run 2D gel.
7. Analyze with any imager capable of detecting Cyanine2, Cyanine3, and Cyanine5. Protein spots can be cut out from the

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gel and analyzed by mass-spectrometry.

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